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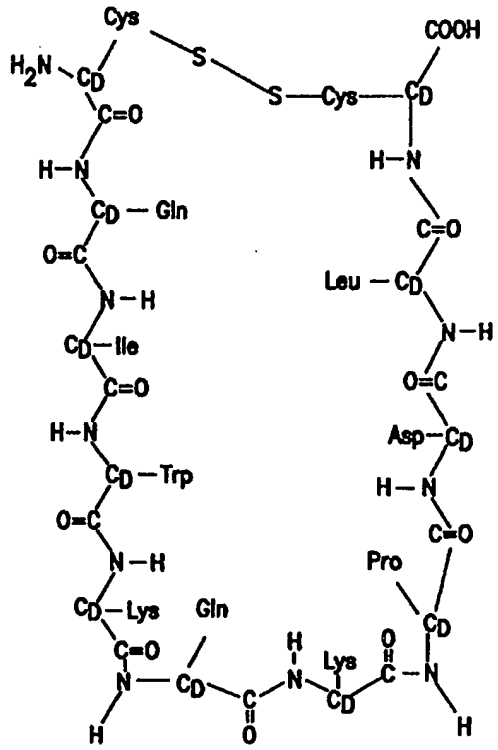
(57) Abstract

Isolated and purified chemokine peptides, variants, and derivatives thereof, as well as chemokine peptide analogs, are provided.



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CHEMOKINE PEPTIDES, VARIANTS, DERIVATIVES AND ANALOGS. THEIR USE IN METHODS TO INHIBIT OR AUGMENT AN INFLAMMATORY RESPONSE

Cross-Reference to Related Applications

This application is a continuation-in-part application of U.S. application Serial No. 08/927,939, filed September 11, 1997, the disclosure of which is incorporated by reference herein.

Background of the Invention

Macrophage/monocyte recruitment plays a role in the morbidity and mortality of a broad spectrum of diseases, including autoimmune diseases, granulomatous diseases, allergic diseases, infectious diseases, osteoporosis and coronary artery disease. For example, in atherosclerosis early during lipid lesion formation, circulating monocytes adhere to the activated endothelium overlying the incipient plaque. Under appropriate conditions, the monocytes then migrate into the developing intima. In the intima, macrophage accumulate lipoprotein and excrete an excess of proteases relative to protease inhibitors. If the lipoproteins are oxidized, they are toxic to macrophage, which results in macrophage death and an increase in an unstable, necrotic, extracellular lipid pool. An excess of proteases results in loss of extracellular matrix and destabilization of the fibrous plaque. Plaque instability is the acute cause of myocardial infarction.

Many molecules have been identified that are necessary for the recruitment of monocytes and other inflammatory cell types. These molecules represent targets for the inhibition of monocyte recruitment. One class of such molecules is adhesion molecules, e.g., receptors, for monocytes. Another class of molecules includes inflammatory mediators, such as TNF-α and related molecules, the interleukins, e.g., IL-1β, and chemokines, e.g., monocyte chemoattractant protein-1 (MCP-1). As a result, agents which modulate the activity of chemokines are likely to be useful to prevent and treat a wide range of diseases. For example, Rollins et al. (U.S. Patent No. 5,459,128) generally disclose analogs of MCP-1 that inhibit the

monocyte chemoattractant activity of endogenous MCP-1. Analogs that are effective to inhibit endogenous MCP-1 are disclosed as analogs which are modified at 28-tyrosine, 24-arginine, 3-aspartate and/or in amino acids between residues 2-8 of MCP-1. In particular, Rollins et al. state that "[s]uccessful inhibition of the activity is found where MCP-1 is modified in one or more of the following ways: a) the 28-tyrosine is substituted by aspartate, b) the 24-arginine is substituted by phenylalanine, c) the 3-aspartate is substituted by alanine, and/or d) the 2-8 amino acid sequence is deleted" (col. 1, lines 49-54). The deletion of amino acids 2-8 of MCP-1 ("MCP-1(Δ2-8)") results in a polypeptide that is inactive, i.e., MCP-1(Δ2-8) is not a chemoattractant (col. 5, lines 22-23). The only effective MCP-1 inhibitor disclosed in Rollins et al. is MCP-1(Δ2-8).

Recent studies suggest that MCP-1(Δ2-8) exhibits a dominant negative effect, i.e., it forms heterodimers with wild-type MCP-1 that cannot elicit a biological effect (Zhang et al., J. Biol. Chem., 269, 15918 (1994); Zhang et al., Mol. Cell. Biol., 15, 4851 (1995)). Thus, MCP-1(Δ2-8) does not exhibit properties of a classic receptor antagonist. Moreover, MCP-1(Δ2-8) is unlikely to be widely useful for inhibition of MCP-1 activity *in vivo*, as MCP-1(Δ2-8) is a large polypeptide with undesirable pharmacodynamic properties. Furthermore, it is unknown whether MCP-1(Δ2-8) is active as a dominant-negative inhibitor of other chemokines associated with inflammation.

Thus, there is a need to identify agents that inhibit or enhance chemokine-induced macrophage and/or monocyte recruitment and which have desirable pharmacodynamic properties. Moreover, there is a need to identify agents that inhibit or enhance chemokine-induced activities of other cell types, such as lymphocytes. Further, there is a need to identify agents that are pan-selective chemokine inhibitors.

Summary of the Invention

The invention provides a therapeutic agent comprising an isolated and purified chemokine peptide, chemokine peptide variant, chemokine analog, or a derivative thereof. Preferably, the therapeutic agent of the invention inhibits the activity of more than one chemokine, although the agent may not inhibit the activity of all chemokines to the same extent. Alternatively, a preferred therapeutic agent of the invention specifically inhibits the activity of one chemokine to a greater extent than other chemokines. Yet another preferred therapeutic agent of the invention mimics the activity of a chemokine, e.g., it acts as an agonist. Thus, therapeutic agents that are chemokine antagonists and agonists are within the scope of the invention. A further preferred therapeutic agent of the invention is an agent that does not inhibit or mimic the activity of a chemokine but binds to or near the receptor for that chemokine, i.e., it is a neutral agent.

A preferred embodiment of the invention is an isolated and purified CC chemokine peptide 3, e.g., a peptide derived from MCP-1 which corresponds to about residue 46 to about residue 67 of mature MCP-1 ("peptide 3[MCP-1]"), a variant, an analog, or a derivative thereof. It is contemplated that chemokine peptide 3, a variant, an analog or a derivative thereof is a chemokine receptor antagonist, although these therapeutic agents may exert their effect by a different mechanism, e.g., by inhibiting the arachidonic acid pathway (e.g., inhibition of leukotriene, thromboxane, or prostaglandin synthesis or stability) or by elevating TGF-beta levels, or by more than one mechanism.

A preferred peptide 3 of the invention is a compound of formula (I):

$$[[(X^2)\text{-}(X^3)\text{-}C\text{-}(X)\text{-}(X^7)\text{-}P]_a\text{-}[(X^4)\text{-}(Z)\text{-}(X^5)\text{-}W\text{-}(X^1)\text{-}(X^6)]_b]_c$$

wherein X^2 is E, Q, D, N, L, P, I or M, wherein X^3 is I, V, M, A, P, norleucine or L, wherein X is A, L, V, M, P, norleucine or I, wherein X^4 is K, S, R, R, Q, N or T, wherein Z is Q, K, E, N, R, I, V, M, A, P, norleucine or L, wherein X^7 is D or P, wherein X^5 is K, E, R, S, Q, D, T, H or N, wherein X^1 is V, L, M, P, A, norleucine, or I, wherein X^6 is Q, N, K or R, wherein a is 0-6, wherein b is 0-6, and wherein c is

1-6, with the proviso that a and b cannot both be 0. The letters in formulas (I)-(III) that are not X, Y or Z represent peptidyl residues as shown in Figure 13. A more preferred peptide 3 of the invention is a compound of formula (I):

$$[[(X^2)\text{-}(X^3)\text{-C-(X)-D-P}]_a\text{-}[(X^4)\text{-(Z)-}(X^5)\text{-W-}(X^1)\text{-}(X^6)]_b]_c$$

wherein X^2 is E, Q or M, wherein X^3 is I, V or L, wherein X is A, L or I, wherein X^4 is K, S or T, wherein Z is Q, K, E or L, wherein X^5 is K, E, R, S or T, wherein X^1 is V or I, wherein X^6 is Q or R, wherein a is 0-6, wherein b is 0-6, and wherein c is 1-6, with the proviso that a and b cannot both be 0.

Yet another preferred peptide 3 of the invention is a compound of formula (II):

$$[[(X^4)\text{-(Z)-}(X^5)]_a\text{-}[W\text{-}(X^1)\text{-}(X^6)]_b]_c$$

wherein X^4 is K, S or T, wherein Z is Q, K, E or L, wherein X^5 is K, E, R, S or T, wherein X^1 is V or I, wherein X^6 is Q or R, wherein a is 0-6, wherein b is 0-6, and wherein c is 1-6, with the proviso that a and b cannot both be 0.

Another preferred peptide 3 of the invention is a compound of formula (II):

$$[[(X^4)\text{-(Z)-}(X^5)]_a\text{-}[W\text{-}(X^1)\text{-}(X^6)]_b]_c$$

wherein X^4 is K, S, R, R, Q, N or T, wherein Z is Q, K, E, N, R, I, V, M, A, P, norleucine or L, wherein X^5 is K, E, R, S, Q, D, T, H or N, wherein X^1 is V, L, M, P, A, norleucine, or I, wherein X^6 is Q, N, K or R, wherein a is 0-6, wherein b is 0-6, and wherein c is 1-6, with the proviso that a and b cannot both be 0.

A more preferred peptide 3 of the invention is a compound of formula (X):

$$(X^8)\text{-(X)-D-}(X^2)\text{-}(X^4)\text{-(Z)-}(X^5)\text{-W-}(X^1)\text{-Q-}(X^7)$$

wherein X is A, L, V or I, wherein X^2 is P, G or L, wherein X^4 is K, T, R or N, wherein Z is Q, K, A or L, wherein X^5 is K, E, R, Q or P, wherein X^1 is V, L, A, M, F or I, and wherein X^8 and X^7 are independently C or absent.

A preferred embodiment of the invention is an isolated and purified CC chemokine peptide 3, e.g., a peptide derived from MCP-1 which corresponds to SEQ ID NO:1 ("peptide 3(1-12)[MCP-1]") or SEQ ID NO:7 ("peptide 3(3-12)[MCP-1]"), a fragment, a variant, an analog, or a derivative thereof. As

described hereinbelow, peptide 3(1-12)[MCP-1](SEQ ID NO:1) and peptide 3(3-12)[MCP-1] (SEQ ID NO:7) are pan-chemokine inhibitors, bioavailable, and have desirable pharmacokinetics. Another preferred CC chemokine peptide 3 of the invention is peptide 3[MIP1α], and more preferably peptide 3(1-12)[MIP1α] which has an amino acid sequence corresponding to SEQ ID NO:42, a variant, an analog, a fragment or a derivative thereof.

Further preferred embodiments of the invention are a CC chemokine peptide 3 such as peptide 3(1-12)[MCP-4] (e.g., SEQ ID NO:65), peptide 3(1-12)[MCP-3](e.g., SEQ ID NO:66), peptide 3(1-12)[MCP-2] (e.g., SEQ ID NO:67), peptide 3(1-12)[eotaxin] (e.g., SEQ ID NO:68), peptide 3(1-12)[MIP1α],(e.g., SEQ ID NO:42), peptide 3(1-12)[MIP1β] (e.g., SEQ ID NO:43), peptide 3(1-12)[RANTES](e.g., SEQ ID NO:44), or a fragment thereof.

Another preferred embodiment of the invention includes a CXC chemokine peptide 3, a variant, an analog or a derivative thereof. A preferred CXC peptide 3 of the invention is a compound of formula (III):

$$[[(X^2)\text{-}(X^3)\text{-}C\text{-}L\text{-}(X)\text{-}(X^7)]_a\text{-}[(X^4)\text{-}(Z)\text{-}(X^5)\text{-}(X^8)\text{-}(X^1)\text{-}(X^6)]_b]_c$$

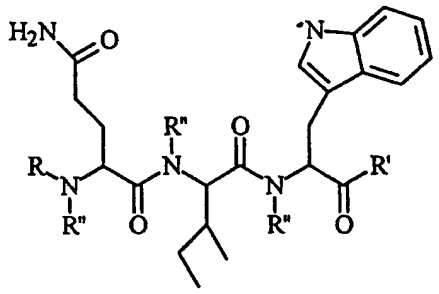
wherein X^2 is E or K, wherein X^3 is I, A, R or L, wherein X is D or N, wherein X^7 is Q, P or L, wherein X^4 is E, K, D, A or Q, wherein Z is A, R, S or E, wherein X^5 is P, N or K, wherein X^8 is F, W, R, I, M, L or A, wherein X^1 is L, V, Y or I, wherein X^6 is K or Q, wherein a is 0-6, wherein b is 0-6, and wherein c is 1-6, with the proviso that a and b cannot both be 0.

Further preferred embodiments of the invention are a CXC chemokine peptide 3 such as peptide 3(1-12)[IL8] (e.g., SEQ ID NO:40), peptide 3(1-12)[SDF-1](e.g., SEQ ID NO:38), peptide 3(1-12)[ENA-78](e.g., SEQ ID NO:41), peptide 3(1-12)[GROα](e.g., SEQ ID NO:72), peptide 3(1-12)[GROβ](e.g., SEQ ID NO:73), peptide 3(1-12)[GROγ](e.g., SEQ ID NO:74), or fragments thereof.

Yet other preferred embodiments of the invention are a CX_2C, CX_3C or C chemokine peptide 3, a variant, an analog or a derivative thereof.

Preferably, a chemokine peptide 3, its variants, analogs or derivatives inhibits the arachidonic acid pathway, e.g., inhibits the synthesis or stability, or binding, of thromboxane, prostaglandin, leukotriene, or any combination thereof.

Other compounds of the invention include compounds of formula (VIII):



wherein

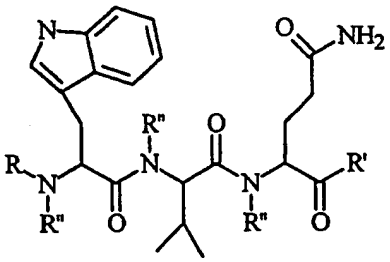
R is (C_1-C_6)alkyl, (C_1-C_6)alkanoyl, aryl, heteroaryl, (C_1-C_6)alkoxycarbonyl, or benzyloxycarbonyl, wherein aryl, heteroaryl, and the phenyl ring of the benzyloxycarbonyl can optionally be substituted with one or more (e.g. 1, 2, 3, or 4), halo, hydroxy, cyano nitro, trifluoromethyl, trifluoromethoxy, (C_1-C_6)alkyl, (C_1-C_6)alkoxy, (C_1-C_6)alkanoyl, (C_2-C_6)alkanoyloxy or (C_1-C_6)alkoxycarbonyl;

R' is (C_1-C_6)alkoxy, aryloxy, or NR_aR_b, wherein R_a and R_b are each independently hydrogen, (C_1-C_6)alkyl, aryl, benzyl, or phenethyl; or R_a, and R_b together with the nitrogen to which they are attached are a 5-6 membered heterocyclic ring (e.g. pyrrolidino, piperidino, or morpholino); and

each R" is independently hydrogen, (C_1-C_6)alkyl, phenyl, benzyl, or phenethyl;

or a pharmaceutically acceptable salt thereof. Preferably, R is benzyloxycarbonyl and R' is dimethylamino or diethylamino, or R is benzyloxycarbonyl; and R' is benzyloxy.

Other compounds of the invention include compounds of formula (IX):



wherein

R is (C_1-C_6)alkyl, (C_1-C_6)alkanoyl, aryl, heteroaryl, (C_1-C_6)alkoxycarbonyl, or benzyloxycarbonyl, wherein aryl, heteroaryl, and the phenyl ring of the benzyloxycarbonyl can optionally be substituted with one or more (e.g. 1, 2, 3, or 4), halo, hydroxy, cyano nitro, trifluoromethyl, trifluoromethoxy, (C_1-C_6)alkyl, (C_1-C_6)alkoxy, (C_1-C_6)alkanoyl, (C_2-C_6)alkanoyloxy or (C_1-C_6)alkoxycarbonyl;

R' is (C_1-C_6)alkoxy, aryloxy, or NR_aR_b, wherein R_a and R_b are each independently hydrogen, (C_1-C_6)alkyl, aryl, benzyl, or phenethyl; or R_a, and R_b together with the nitrogen to which they are attached are a 5-6 membered heterocyclic ring (e.g. pyrrolidino, piperidino, or morpholino); and

each R" is independently hydrogen, (C_1-C_6)alkyl, phenyl, benzyl, or phenethyl;

or a pharmaceutically acceptable salt thereof. Preferably, R is benzyloxycarbonyl and R' is dimethylamino or diethylamino, or R is benzyloxycarbonyl; and R' is benzyloxy.

Another preferred embodiment of the invention includes a chemokine peptide 3 that is at least a tripeptide, a variant thereof or a derivative thereof. A preferred embodiment of the invention is the MCP-1 tripeptide KQK (i.e., peptide 3(9-12)[MCP-1], which specifically inhibits MCP-1, but not MIP1α, IL8 and SDF1α, chemokine-induced activity. Other preferred embodiments of the invention include isolated and purified chemokine tripeptides that specifically inhibit IL8, MIP1α, SDF1, murine MCP-1, MCP-2, MCP-3, and MIP1β, e.g., KEN, SEE, KLK, KKE, KER, TQK, and SES, respectively. A further preferred embodiment of the

invention is a chemokine peptide 3 tripeptide that inhibits the activity of more than one chemokine, e.g., WVQ or WIQ. Preferably, a tripeptide of the invention is not RFK.

Yet another embodiment of the invention is a peptide which includes the amino acid sequence KXK, wherein X is an amino acid, preferably one of the twenty naturally occurring amino acids, and which peptide is a chemokine antagonist, activates TGF-beta (TGF-beta1, TGF-beta2, TGF-beta3, or a combination thereof), or a combination thereof. Preferably, the peptide increases the activation of TGF-beta1. It is preferred that a peptide which includes the amino acid sequence KXK is less than about 15, preferably about 10, and more preferably about 8 amino acid residues in length. Preferably, the peptide is not KKFK or RKPK. A further embodiment of the invention is a peptide which includes a basic amino acid residue followed by phenylalanine followed by another basic residue, wherein the peptide is not RFK, is not KRFK, or does not contain RFK or KRFK.

Another preferred peptide of the invention is a compound of formula (VII):

$$(X^{1})\text{-}(Y)\text{-}(K)\text{-}(X^{2})\text{-}K\text{-}(X^{3})$$

wherein X^2 is V, A, D, E, P, R, C, H, M, F, K, L, N, Q, Y, or I; wherein Y is absent or is an amino acid that is not R or K; and wherein X^1 and X^3 are independently 0-20 amino acid residues or absent. Preferably, X^2 is F, K, L, N, Q, Y, or I. More preferably, X^2 is F, K, L, N, Q, Y, or I, and Y, X^1 and X^3 are absent.

To identify a peptide of the invention useful in the methods of the invention, a sequence comparison of chemokines from different species is performed. Then the cross-reactivity of a non-human chemokine for the human receptor is assessed. A preferred chemokine is one from a species which has the least sequence homology to the corresponding human chemokine, but which still cross-reacts by binding to the human receptor. Regions which have a high degree of sequence similarity or identity are employed to prepare a peptide of the invention. For example, to identify peptides of TGF-beta having antagonist, agonist or neutral properties, the amino acid sequence of human TGF-beta was compared to that of *Xenopus*.

Peptides identified by this method include LYIDFRQDLGWKW ("T1"; SEQ ID NO:111); HEPKGYHANFC ("T2"; SEQ ID NO:112); VYYVGRK ("T3"; SEQ ID NO:113) and KVEQLSNMVVKSC ("T4"; SEQ ID NO:114). Biotinylated T1 bound to the TGF-beta receptor of THP-1 cells with an ED_{50} of 18 μM and is a receptor neutral agent (i.e., neither agonist nor antagonist). Biotinylated T2 bound to the TGF-beta receptor of THP-1 cells with an ED_{50} of 30 μM and is a weak receptor antagonist.

Another preferred embodiment of the invention is an isolated and purified CC chemokine peptide 2, such as a peptide corresponding to SEQ ID NO:3 ("peptide 2(1-15)[MCP-1]"), SEQ ID NO:5 ("peptide 2(1-14)[MIP1α]"), a fragment, a variant, an analog, or a derivative thereof. It is contemplated that chemokine peptide 2, a variant, an analog or a derivative thereof is a chemokine receptor agonist, although these therapeutic agents may exert their effect by a different mechanism, or by more than one mechanism. It is also envisioned that chemokine peptide 2, a variant, an analog or a derivative thereof is a chemokine receptor antagonist. Preferably, a variant, an analog or a derivative of peptide 2 has reduced Duffy antigen binding, and also preferably, enhanced receptor binding properties, relative to the corresponding peptide 2 having a native or wild-type amino acid sequence.

Other preferred CC chemokine peptides 2 include peptide 2(1-14)[MIP1β] (e.g., SEQ ID NO:60), peptide 2(1-15)[RANTES] (e.g., SEQ ID NO:61), peptide 2(1-15)[MCP-2] (e.g., SEQ ID NO:62), peptide 2(1-15)[MCP-3](e.g., SEQ ID NO:63), peptide 2(1-15)[MCP-4] (e.g., SEQ ID NO:64), peptide 2(1-15)[eotaxin] (e.g., SEQ ID NO:75), or a fragment thereof.

Another preferred embodiment of the invention includes a CXC chemokine peptide 2, a variant, an analog or a derivative thereof. Preferred CC chemokine peptide 2 includes peptide 2(1-15)[IL8] (e.g., SEQ ID NO:6), peptide 2(1-15)[SDF1 (e.g., SEQ ID NO:4), peptide 2(1-15)[ENA78] (e.g., SEQ ID NO:59), peptide 2(1-

15)[GROα] (e.g., SEQ ID NO:69), peptide 2(1-15)[GROβ](e.g., SEQ ID NO:70), peptide 2(1-15)[GROγ] (e.g., SEQ ID NO:71), or a fragment thereof.

Yet another preferred embodiment of the invention is a CX_2C, CX_3C or C chemokine peptide 2, a variant, an analog or a derivative thereof.

Other preferred peptide 2s include TSSKC (peptide 2(1-5)[MIP-1]; SEQ ID NO:102); DYFETSSQC (peptide 2(1-9)[MIP1α]; SEQ ID NO:103); CSKPGV (peptide 2(9-14)[MIP1α]; SEQ ID NO:104); HLKILNTPNCALQIV (peptide 2(1-5)[MIP-1α]; SEQ ID NO:105); SYRRITSSK (peptide 2(1-5)[MCP-1]; SEQ ID NO:106); CPKEAV (peptide 2(10-15)[MCP-1]; SEQ ID NO:107); SYRRI (peptide 2(1-5)[MCP-1]; SEQ ID NO:108); and CSYRRITSSKSPKEAVC (SEQ ID NO:110); as well as a peptide having D isomers of the sequence VGPKSCQSSTEFYD (corresponding to residues 1-14 of peptide 2(1-14)[MIP1α], lowercase letters are used herein to indicate D isomers as well as the letter "D" in CRD and LRD); a peptide corresponding to vaekpcksstirry; and a variant peptide 2 of vgpkscqsstefyd (LRD peptide 2(1-14)[MIP1α] which includes a D isomer of serine at position 10, and the D isomer of cysteine at the amino and carboxy termini of the peptide (designated LRD-$Cys_0Ser_{10}Cys_{16}$ peptide 2(1-15)[MCP-1], where L = linear, F = forward, D = D isomer).

A more preferred peptide 2 of the invention is a compound of formula (XII):

C-(X)-Y-(X^2)-(X^4)-(Z)-T-(X^5)-(X^6)-(X^1)-C-(X^8)-(X^7)-(X^9)-(X^{10})-V-C

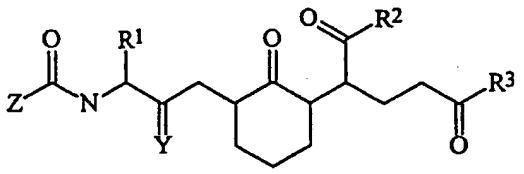
wherein X is S or D, wherein X^2 is R, K, F or Y, wherein X^4 is R, E or F, wherein Z is T or a peptide bond, wherein X^5 is S or N, wherein X^6 is S or I, wherein X^1 is K, R, Q or L, wherein X^8 is P or S, wherein X^7 is K, R or Q, wherein X^9 is E or P, and wherein X^{10} is A or G.

Also provided is an isolated and purified chemokine peptide variant, or a derivative thereof. A chemokine peptide variant has at least about 50%, preferably at least about 80%, and more preferably at least about 90% but less than 100%, contiguous amino acid sequence homology or identity to the amino acid sequence of the corresponding native chemokine, e.g., Ser_7 peptide 3(1-12)[MCP1] (SEQ ID

NO:11) has less than 100% contiguous homology to the corresponding amino acid sequence of MCP-1, i.e., a peptide having SEQ ID NO:1. A preferred peptide 3 variant is Leu_4Ile_{11}peptide 3(3-12)[MCP-1].

The invention also provides derivatives of chemokine peptides and peptide variants. A preferred derivative is a cyclic reverse sequence D isomer (CRD) derivative of a chemokine peptide, a variant or an analog thereof of the invention. For example, LRD derivatives of peptide 2, CRD-$Cys_0Ser_{10}Cys_{16}$ peptide 2[MCP-1] and CRD-$Cys_{13}Leu_4Ile_{11}$peptide 3(3-12)[MCP-1] are aompounds of the invention that are particularly useful in the practice of the methods of the invention, as described hereinbelow.

Also provided are certain analogs of chemokines. In particular, analogs of chemokine peptide 2, chemokine peptide 3, or variants thereof are contemplated. A preferred analog of chemokine peptide 3 is an analog of WIQ. Thus, a preferred chemokine analog of the invention includes a compound of formula (IV):



(IV)

wherein R^1 is aryl, heteroaryl, aryl(C_1-C_3)alkyl, heteroaryl(C_1-C_3)alkyl, coumaryl, coumaryl(C_1-C_3)alkyl, chromanyl or chromanyl(C_1-C_3)alkyl; wherein any aryl or heteroaryl group, or the benz-ring of any coumaryl or chromanyl group may optionally be substituted with one, two or three substituents selected from the group consisting of halo, nitro, cyano, hydroxy, (C_1-C_6)alkyl, (C_1-C_6)alkoxy, (C_1-C_6)alkanoyl, (C_2-C_6)alkanoyloxy, -C(=O)(C_1-C_6)alkoxy, C(=O)NR^gR^h, NR^iR^j;

wherein R^2 is (C_1-C_{10})alkyl, (C_3-C_6)cycloalkyl, (C_3-C_6)cycloalkyl(C_1-C_6)alkyl, (C_1-C_{10})alkoxy, (C_3-C_6)cycloalkyl(C_1-C_6)alkoxy or $N(R^a)(R^b)$;

wherein R^3 is $(C_1\text{-}C_{10})$alkyl, $(C_3\text{-}C_6)$cycloalkyl, $(C_3\text{-}C_6)$cycloalkyl$(C_1\text{-}C_6)$alkyl, $(C_1\text{-}C_{10})$alkoxy, $(C_3\text{-}C_6)$cycloalkyl$(C_1\text{-}C_6)$alkoxy or $N(R^c)(R^d)$;

wherein Y is oxo or thioxo;

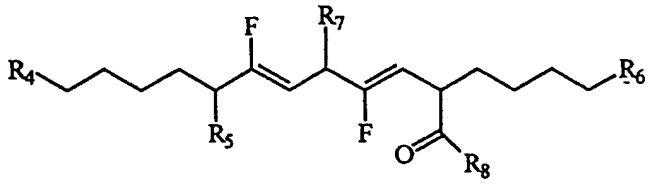
wherein Z is $(C_1\text{-}C_{10})$alkyl, $(C_3\text{-}C_6)$cycloalkyl, $(C_3\text{-}C_6)$cycloalkyl$(C_1\text{-}C_6)$alkyl, $(C_1\text{-}C_{10})$alkoxy, $(C_3\text{-}C_6)$cycloalkyl$(C_1\text{-}C_6)$alkoxy or $N(R^e)(R^f)$; and

wherein R^a-R^j are each independently hydrogen, $(C_1\text{-}C_{10})$alkyl, $(C_1\text{-}C_{10})$alkanoyl, phenyl, benzyl, or phenethyl; or R^a and R^b, R^c and R^d, R^e and R^f, R^g and R^h, or R^i and R^j together with the nitrogen to which they are attached form a ring selected from pyrrolidino, piperidino, or morpholino; or a pharmaceutically acceptable salt thereof.

A preferred embodiment of a compound of formula (IV) includes a compound of a formula (IV) wherein R^1 is aryl, heteroaryl, coumaryl, or chromanyl. Preferably aryl is phenyl; and heteroaryl is indolyl or pyridinyl. Another preferred embodiment of a compound of formula (IV) includes a compound of a formula (IV) wherein R^2 is $N(R^a)(R^b)$; and R^3 is $N(R^c)(R^d)$. Yet another preferred embodiment of a compound of formula (IV) includes a compound of a formula (IV) wherein Z is $(C_1\text{-}C_{10})$alkyl.

A further preferred compound is a compound of formula (IV) wherein R^1 is indolyl; R^2 is $N(R^a)(R^b)$; R^3 is $N(R^c)(R^d)$; Y is S; Z is hydrogen; and R^a, R^b, R^c, and R^d are each methyl.

Another preferred analog of chemokine peptide 3 is an analog of KXK. Thus, the invention includes a compound of formula (V):



wherein

R^4 is NR_kR_l;

R^5 is NR_mR_n;

R^6 is NR_oR_p;

R^7 is the side chain of a natural or unnatural amino acid or is $-(CH_2)_2C(=O)NR_qR_r$;

R^8 is hydrogen, hydroxy, (C_1-C_{10})alkyl, (C_3-C_6)cycloalkyl, (C_3-C_6)cycloalkyl(C_1-C_6)alkyl, (C_1-C_{10})alkoxy, (C_3-C_6)cycloalkyl(C_1-C_6)alkoxy, NR_sR_t, the amino terminus of an amino acid or the N-terminal residue of a peptide of 2 to about 25 amino acid residues;

R_k, R_l, R_o, and R_p are each independently hydrogen, (C_1-C_{10})alkyl, (C_3-C_6)cycloalkyl, (C_3-C_6)cycloalkyl(C_1-C_6)alkyl, (C_1-C_{10})alkanoyl, phenyl, benzyl or phenethyl;

R_m are R_n are each independently hydrogen, (C_1-C_{10})alkyl, (C_3-C_6)cycloalkyl, (C_3-C_6)cycloalkyl(C_1-C_6)alkyl, (C_1-C_{10})alkoxy, (C_1-C_{10})alkanoyl, (C_1-C_{10})alkoxycarbonyl, 9-fluorenylmethoxycarbonyl, phenyl, benzyl, phenethyl, the C-terminal residue of an amino acid or a peptide of 2 to about 25 amino acid residues;

R_q are R_r are each independently hydrogen, (C_1-C_{10})alkyl, (C_3-C_6)cycloalkyl, (C_3-C_6)cycloalkyl(C_1-C_6)alkyl, phenyl, benzyl or phenethyl;

wherein R_s are R_t are each independently hydrogen, (C_1-C_{10})alkyl, (C_3-C_6)cycloalkyl, (C_3-C_6)cycloalkyl(C_1-C_6)alkyl, phenyl, benzyl or phenethyl; or a pharmaceutically acceptable salt thereof.

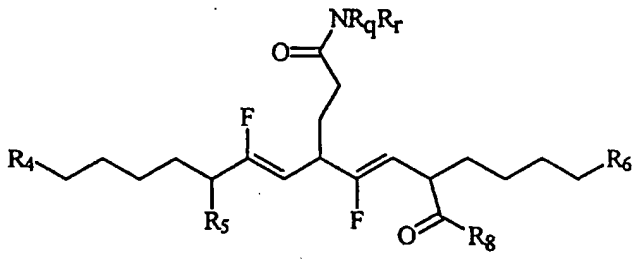
Preferably R_k, R_l, R_o, and R_p are each hydrogen; R_m are R_n are each independently hydrogen, acetyl, (C_1-C_{10})alkyl, (C_3-C_6)cycloalkyl, propoxy, butoxy, *tert*-butoxycarbonyl, 9-fluorenylmethoxycarbonyl or the C-terminal residue of an amino acid or a peptide of 2 to about 25 amino acid residues; and R_q are R_r are each independently hydrogen, (C_1-C_{10})alkyl, or (C_3-C_6)cycloalkyl.

Preferably, R^7 is $-(CH_2)_2C(=O)NR_qR_r$.

Preferably, R^7 is methyl, 3-guanidinopropyl, aminocarbonylmethyl, carboxymethyl, mercaptomethyl, (2-carboxy-2-aminoethyl)dithiomethyl, 2-carboxyethyl, 2-(aminocarbonyl)ethyl, hydrogen, 5-imadazoylmethyl, 4-amino-3-hydroxypropyl, 2-butyl, 2-methylprop-1-yl, 4-aminobutyl, 2-(methylthio)ethyl, benzyl, hydroxymethyl, 1-hydroxyethyl, 3-indolylmethyl, 4-hydroxybenzyl, or isopropyl.

More preferably, R^7 is hydrogen, benzyl, 4-hydroxybenzyl, methyl, 2-hydroxymethyl, or mercaptomethyl.

A preferred compound of formula (V) includes an analog of KGK, KFK, KYK, KAK, KSK, KCK or KQK. For example, an analog of KQK includes a compound of formula (V):



wherein R^4 is NR_kR_l;

wherein R^5 is NR_mR_n;

wherein R^6 is NR_oR_p;

wherein R^7 is NR_qR_r;

wherein R^8 is hydrogen, hydroxy, $(C_1\text{-}C_{10})$alkyl, $(C_3\text{-}C_6)$cycloalkyl, $(C_3\text{-}C_6)$cycloalkyl$(C_1\text{-}C_6)$alkyl, $(C_1\text{-}C_{10})$alkoxy, $(C_3\text{-}C_6)$cycloalkyl$(C_1\text{-}C_6)$alkoxy, NR_sR_t, the amino terminus of an amino acid or the N-terminal residue of a peptide of 2 to about 25 amino acid residues;

wherein R_k, R_l, R_o, and R_p are each independently hydrogen, $(C_1\text{-}C_{10})$alkyl, $(C_3\text{-}C_6)$cycloalkyl, $(C_3\text{-}C_6)$cycloalkyl$(C_1\text{-}C_6)$alkyl, $(C_1\text{-}C_{10})$alkanoyl, phenyl, benzyl or phenethyl;

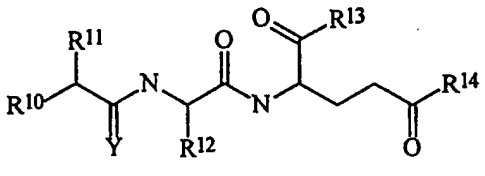
wherein R_m are R_n are each independently hydrogen, $(C_1$-$C_{10})$alkyl, $(C_3$-$C_6)$cycloalkyl, $(C_3$-$C_6)$cycloalkyl$(C_1$-$C_6)$alkyl, $(C_1$-$C_{10})$alkoxy, $(C_1$-$C_{10})$alkanoyl, $(C_1$-$C_{10})$alkoxycarbonyl, 9-fluorenylmethoxycarbonyl, phenyl, benzyl, phenethyl, the C-terminal residue of an amino acid or a peptide of 2 to about 25 amino acid residues;

wherein R_q are R_r are each independently hydrogen, $(C_1$-$C_{10})$alkyl, $(C_3$-$C_6)$cycloalkyl, $(C_3$-$C_6)$cycloalkyl$(C_1$-$C_6)$alkyl, phenyl, benzyl or phenethyl;

wherein R_s are R_t are each independently hydrogen, $(C_1$-$C_{10})$alkyl, $(C_3$-$C_6)$cycloalkyl, $(C_3$-$C_6)$cycloalkyl$(C_1$-$C_6)$alkyl, phenyl, benzyl or phenethyl; or a pharmaceutically acceptable salt thereof.

Preferably R_k, R_l, R_o, and R_p are each hydrogen; R_m are R_n are each independently hydrogen, acetyl, $(C_1$-$C_{10})$alkyl, $(C_3$-$C_6)$cycloalkyl, propoxy, butoxy, *tert*-butoxycarbonyl, 9-fluorenylmethoxycarbonyl or the C-terminal residue of an amino acid or a peptide of 2 to about 25 amino acid residues; and R_q are R_r are each independently hydrogen, $(C_1$-$C_{10})$alkyl, or $(C_3$-$C_6)$cycloalkyl.

Another preferred analog of chemokine peptide 3 is an analog of WVQ (see Figure 8). Thus, the invention provides a compound of formula (VI):



(VI)

wherein

R^{10} is NR^iR^j;

R^{11} is aryl, heteroaryl, aryl$(C_1$-$C_3)$alkyl, heteroaryl$(C_1$-$C_3)$alkyl, coumaryl, coumaryl$(C_1$-$C_3)$alkyl, chromanyl or chromanyl$(C_1$-$C_3)$alkyl; wherein any aryl or heteroaryl group, or the benz-ring of any coumaryl or chromanyl group may optionally be substituted with one, two or three substituents selected from the group consisting of halo, nitro, cyano, hydroxy, $(C_1$-$C_6)$alkyl, $(C_1$-$C_6)$alkoxy, $(C_1$-$C_6)$alkanoyl, $(C_2$-$C_6)$alkanoyloxy, -C(=O)$(C_1$-$C_6)$alkoxy, C(=O)NR^gR^h, NR^eR^f;

R^{12} is $(C_1$-$C_6)$alkyl;

R^{13} is (C_1-C_{10})alkyl, (C_3-C_6)cycloalkyl, (C_3-C_6)cycloalkyl(C_1-C_6)alkyl, (C_1-C_{10})alkoxy, (C_3-C_6)cycloalkyl(C_1-C_6)alkoxy, hydroxy, or $N(R^a)(R^b)$;

R^{14} is (C_1-C_{10})alkyl, (C_3-C_6)cycloalkyl, (C_3-C_6)cycloalkyl(C_1-C_6)alkyl, (C_1-C_{10})alkoxy, (C_3-C_6)cycloalkyl(C_1-C_6)alkoxy or $N(R^c)(R^d)$;

Y is oxo or thioxo;

wherein R^a-R^j are each independently hydrogen, (C_1-C_{10})alkyl, (C_1-C_{10})alkanoyl, phenyl, benzyl, or phenethyl; or R^a and R^b, R^c and R^d, R^e and R^f, R^g and R^h, or R^i and R^j together with the nitrogen to which they are attached form a ring selected from pyrrolidino, piperidino, or morpholino; or a pharmaceutically acceptable salt thereof.

Preferably, R^{10} is amino; R^{11} is 2-benzimidazolyl; R^{12} is (C_1-C_6)alkyl; R^{13} is hydroxy; and R^{14} is amino.

It is envisioned that the therapeutic agents of the invention include compounds having a chiral center that can be isolated in optically active and racemic forms.

Further provided are isolated and purified nucleic acid molecules, e.g., DNA molecules, comprising a preselected nucleic acid segment which encodes at least a portion of a chemokine, i.e., they encode a chemokine peptide or a variant thereof as described herein, e.g., a chemokine 3 peptide, a variant or derivative thereof or a chemokine peptide 2, a variant or derivative thereof. For example, the invention provides an expression cassette comprising a preselected DNA segment which codes for an RNA molecule which is substantially identical (sense) to all or a portion of a messenger RNA ("target" mRNA), i.e., an endogenous or "native" chemokine mRNA. The preselected DNA segment in the expression cassette is operably linked to a promoter. As used herein, "substantially identical" in sequence means that two nucleic acid sequences have at least about 65%, preferably about 70%, more preferably about 90%, and even more preferably about 98%, contiguous nucleotide sequence identity to each other. Preferably, the preselected DNA segment hybridizes under hybridization conditions, preferably under stringent hybridization

conditions, to a nucleic acid molecule encoding the corresponding native chemokine.

The present invention also provides isolated and purified DNA molecules which provide "anti-sense" mRNA transcripts of the DNA segments that encode a chemokine which, when expressed from an expression cassette in a host cell, can alter chemokine expression. As used herein, the term "antisense" means a sequence of nucleic acid which is the reverse complement of at least a portion of a RNA molecule that codes for a chemokine. Preferably, the antisense sequences of the invention are substantially complementary to a DNA segment encoding a peptide or peptide variant of the invention. As used herein, "substantially complementary" means that two nucleic acid sequences have at least about 65%, preferably about 70%, more preferably about 90%, and even more preferably about 98%, contiguous nucleotide sequence complementarity to each other. A substantially complementary RNA molecule is one that has sufficient sequence complementarity to the mRNA encoding a chemokine so as to result in a reduction or inhibition of the translation of the mRNA. It is envisioned that the duplex formed by the antisense sequence and the mRNA inhibits translation of the mRNA, as well as promotes RNA degradation, although anti-sense sequences may exert their effect by other mechanisms, or by a combination of mechanisms. Preferably, the preselected antisense DNA segment hybridizes under hybridization conditions, preferably under stringent hybridization conditions, to a nucleic acid molecule comprising the corresponding chemokine gene.

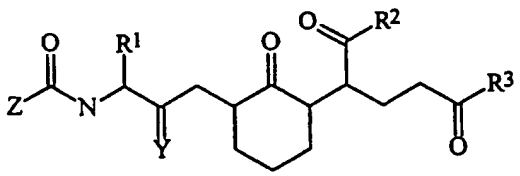
The introduction of chemokine sense or antisense nucleic acid into a cell *ex vivo* or *in vivo* can result in a molecular genetic-based therapy directed to controlling the expression of the chemokine. Thus, the introduced nucleic acid may be useful to correct or supplement the expression of the gene in patients with a chemokine-associated indication. For example, the administration of an expression vector encoding a peptide of the invention which is a chemokine receptor agonist may increase the chemokine signaling and thus be efficacious for diseases which are

characterized by decreased levels of the chemokine. Likewise, the administration of an expression vector comprising antisense chemokine sequences may be useful to prevent or treat a disorder associated with increased chemokine expression. For example, an expression vector containing antisense peptide 3(1-12)[MCP-1] which is introduced into the lungs may be efficacious to prevent or treat asthma.

Also provided are pharmaceutical compositions, delivery systems, and kits comprising the therapeutic agents of the invention.

The invention further provides methods to treat chemokine-associated indications. For example, the invention provides a method of preventing or inhibiting an indication associated with chemokine-induced activity. The method comprises administering to a mammal afflicted with, or at risk of, the indication an amount of a chemokine peptide 3, a chemokine peptide 2, a fragment thereof, a variant thereof, a derivative thereof, a compound of formula (VII), a compound of formula (VIII), a compound of formula (IX), a compound of formula (X), a compound of formula (XI), a compound of formula (XII), or a combination thereof, effective to prevent or inhibit said activity. Preferably, the peptide is not an IL-8 peptide, a NAP-2 peptide, or a PF4 peptide. Preferably, the administration is effective to inhibit the activity of more than one chemokine (i.e., the peptide is a pan-selective inhibitor). Preferred pan-chemokine inhibitors are WVQ, WIQ, Leu_4Ile_{11}peptide 3(3-12)[MCP-1], Leu_4Ile_{11}peptide 3(1-12)[MCP-1] and CRD-$Cys_{13}Leu_4Ile_{11}$peptide 3(3-12). These agents are useful to treat indications such as multiple sclerosis, asthma, psoriasis, allergy, rheumatoid arthritis, organ transplant rejection, and autoimmune disorders. Preferred chemokine peptides useful to treat or inhibit these indications include peptide 2 and/or peptide 3 from MCP-1, MCP-2, MCP-3, MCP-4, RANTES, MIP1α, ENA78, MIG, GROβ, eotaxin, IP10, MIPβ and SDF-1.

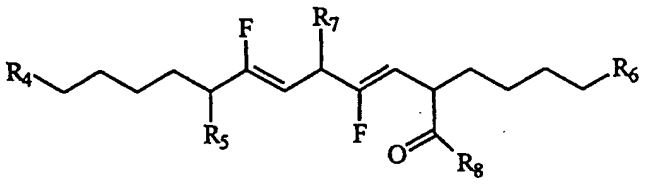
The invention also provides a method of treating a mammal afflicted with, or at risk of, an indication associated with chemokine-induced activity, comprising: administering to the mammal an effective amount of a compound of formula (IV):



(IV)

wherein R^1 is aryl, heteroaryl, coumaryl or chromanyl; wherein R^2 is $N(R^a)(R^b)$; wherein R^3 is $N(R^c)(R^d)$; wherein Y is oxo or thioxo; wherein Z is $(C_1\text{-}C_{10})$alkyl; wherein R^a-R^d are each independently hydrogen, $(C_1\text{-}C_{10})$alkyl, $(C_1\text{-}C_{10})$alkanoyl, phenyl, benzyl or phenethyl; or wherein R^a and R^b, or R^c and R^d, together with the nitrogen to which they are attached form a pyrrolidino, piperidino or morpholino ring; or a pharmaceutically acceptable salt thereof.

Further provided is a method of treating a mammal afflicted with, or at risk of, an indication associated with chemokine-induced activity, comprising: administering to the mammal an effective amount of a compound of formula (V):

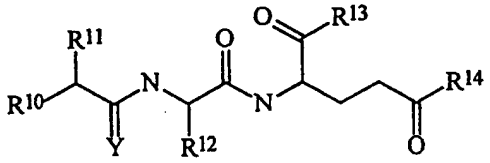


wherein R^4 is NR_kR_l; R^5 is NR_mR_n; R^6 is NR_oR_p; R^7 is the side chain of a natural or unnatural amino acid or is $-(CH_2)_2C(=O)NR_qR_r$; R^8 is hydrogen, hydroxy, $(C_1\text{-}C_{10})$alkyl, $(C_3\text{-}C_6)$cycloalkyl, $(C_3\text{-}C_6)$cycloalkyl$(C_1\text{-}C_6)$alkyl, $(C_1\text{-}C_{10})$alkoxy, $(C_3\text{-}C_6)$cycloalkyl$(C_1\text{-}C_6)$alkoxy, NR_sR_t, the amino terminus of an amino acid or the N-terminal residue of a peptide of 2 to about 25 amino acid residues; R_k, R_l, R_o, and R_p are each independently hydrogen, $(C_1\text{-}C_{10})$alkyl, $(C_3\text{-}C_6)$cycloalkyl, $(C_3\text{-}C_6)$cycloalkyl$(C_1\text{-}C_6)$alkyl, $(C_1\text{-}C_{10})$alkanoyl, phenyl, benzyl or phenethyl; R_m are R_n are each independently hydrogen, $(C_1\text{-}C_{10})$alkyl, $(C_3\text{-}C_6)$cycloalkyl, $(C_3\text{-}C_6)$cycloalkyl$(C_1\text{-}C_6)$alkyl, $(C_1\text{-}C_{10})$alkoxy, $(C_1\text{-}C_{10})$alkanoyl, $(C_1\text{-}$

C_{10})alkoxycarbonyl, 9-fluorenylmethoxycarbonyl, phenyl, benzyl, phenethyl, the C-terminal residue of an amino acid or a peptide of 2 to about 25 amino acid residues; R_q are R_r are each independently hydrogen, (C_1-C_{10})alkyl, (C_3-C_6)cycloalkyl, (C_3-C_6)cycloalkyl(C_1-C_6)alkyl, phenyl, benzyl or phenethyl; wherein R_s are R_t are each independently hydrogen, (C_1-C_{10})alkyl, (C_3-C_6)cycloalkyl, (C_3-C_6)cycloalkyl(C_1-C_6)alkyl, phenyl, benzyl or phenethyl; or a pharmaceutically acceptable salt thereof.

Also provided is a method of treating a mammal afflicted with, or at risk of, an indication associated with chemokine-induced activity, comprising:

administering to the mammal an effective amount of a compound of formula (VI):



(VI)

wherein

R^{10} is NR^iR^j;

R^{11} is aryl, heteroaryl, aryl(C_1-C_3)alkyl, heteroaryl(C_1-C_3)alkyl, coumaryl, coumaryl(C_1-C_3)alkyl, chromanyl or chromanyl(C_1-C_3)alkyl; wherein any aryl or heteroaryl group, or the benz-ring of any coumaryl or chromanyl group may optionally be substituted with one, two or three substituents selected from the group consisting of halo, nitro, cyano, hydroxy, (C_1-C_6)alkyl, (C_1-C_6)alkoxy, (C_1-C_6)alkanoyl, (C_2-C_6)alkanoyloxy, -C(=O)(C_1-C_6)alkoxy, C(=O)NR^gR^h, NR^eR^f;

R^{12} is (C_1-C_6)alkyl;

R^{13} is (C_1-C_{10})alkyl, (C_3-C_6)cycloalkyl, (C_3-C_6)cycloalkyl(C_1-C_6)alkyl, (C_1-C_{10})alkoxy, (C_3-C_6)cycloalkyl(C_1-C_6)alkoxy, hydroxy, or $N(R^a)(R^b)$;

R^{14} is (C_1-C_{10})alkyl, (C_3-C_6)cycloalkyl, (C_3-C_6)cycloalkyl(C_1-C_6)alkyl, (C_1-C_{10})alkoxy, (C_3-C_6)cycloalkyl(C_1-C_6)alkoxy or $N(R^c)(R^d)$;

Y is oxo or thioxo;

wherein R^a-R^j are each independently hydrogen, (C_1-C_{10})alkyl, (C_1-C_{10})alkanoyl, phenyl, benzyl, or phenethyl; or R^a and R^b, R^c and R^d, R^e and R^f, R^g

and R^h or R^i and R^j together with the nitrogen to which they are attached form a ring selected from pyrrolidino, piperidino, or morpholino; or a pharmaceutically acceptable salt thereof.

The invention further provides a method to increase, augment or enhance a chemokine-associated inflammatory response in a mammal, comprising: administering to the mammal an amount of a chemokine peptide 3, a chemokine peptide 2, a variant thereof, a derivative thereof, a compound of formula (IV), a compound of formula (V), a compound of formula (VI), a compound of formula (VII), a compound of formula (VIII), a compound of formula (IX), a compound of formula (X), a compound of formula (XI), a compound of formula (XII), or a combination thereof, effective to increase, augment or enhance said response. Moreover, as peptide 3, its variants and derivatives may decrease Th2 responses and increase Th1 responses, these compounds may be particularly useful to treat or prevent specific diseases in which a decrease in Th2 response and an increase in Th1 response is indicated. It is preferred that the agent employed to increase, augment or enhance the chemokine-associated inflammatory response is not YNFTNRKISVQRLASYRRITSSK. These therapeutic agents are useful to increase an inflammatory response to, for example, intracellular pathogens or parasites, which often are associated with a poor immune response. Thus, these agents may be useful to treat or prevent tuberculosis and malaria. Therefore, the invention also provides a therapeutic method to prevent or treat parasitic infection.

The invention also provides a method of preventing or inhibiting an indication associated with histamine release from basophils or mast cells. The method comprises administering to a mammal at risk of, or afflicted with, the indication an effective amount of a chemokine peptide 3, a chemokine peptide 2, a variant thereof, a derivative thereof, a compound of formula (IV), a compound of formula (V), a compound of formula (VI), a compound of formula (VII), a compound of formula (VIII), a compound of formula (IX), a compound of formula

(X), a compound of formula (XI), a compound of formula (XII), or a combination thereof.

Also provided is a method of preventing or inhibiting an indication associated with monocyte, macrophage, neutrophil, B cell, T cell or eosinophil recruitment, or B cell or T cell activation or proliferation. The method comprises administering an effective amount of a chemokine peptide 3, a chemokine peptide 2, a variant thereof, a derivative thereof, a compound of formula (IV), a compound of formula (V), a compound of formula (VI), a compound of formula (VII), a compound of formula (VIII), a compound of formula (IX), a compound of formula (X), a compound of formula (XI), a compound of formula (XII), or a combination thereof. For example, a chemokine peptide 3, a chemokine peptide 2, a variant thereof, or a derivative thereof may be useful to prevent or treat autoimmune or granulomatous indications.

Further provided is a therapeutic method to prevent or treat vascular indications, comprising: administering to a mammal in need of such therapy an effective amount of a chemokine peptide 3, a chemokine peptide 2, a variant thereof, a derivative thereof, a compound of formula (IV), a compound of formula (V), a compound of formula (VI), a compound of formula (VII), a compound of formula (VIII), a compound of formula (IX), a compound of formula (X), a compound of formula (XI), a compound of formula (XII), or a combination thereof, wherein the indication is coronary artery disease, myocardial infarction, unstable angina pectoris, atherosclerosis or vasculitis. Preferred chemokine peptides for this embodiment of the invention include chemokine peptides of MCP-1, RANTES, GROα, MIP1α, IP10, MCP-4, and MIP1β.

The invention also provides a method to prevent or treat an autoimmune disorder. The method comprises administering to a mammal in need of said therapy an effective amount of a chemokine peptide 3, a chemokine peptide 2, a variant thereof, a derivative thereof, a compound of formula (IV), a compound of formula (V), a compound of formula (VI), a compound of formula (VII), a

compound of formula (VIII), a compound of formula (IX), a compound of formula (X), a compound of formula (XI), a compound of formula (XII), or a combination thereof. A preferred variant of peptide 3 useful to prevent or treat autoimmune disorders is Leu_4Ile_{11}peptide 3(1-12)[MCP-1] (SEQ ID NO:14) or peptide 3 having WVQ. A preferred chemokine peptide 3 for use in preventing or treating multiple sclerosis includes SEE and peptide 3(1-14)[MIP1α] (SEQ ID NO:42). Other preferred peptides are chemokine peptides of RANTES.

Further provided is a method to modulate the chemokine-induced activity of macrophage, B cells, T cells or other hematopoietic cells, e.g., neutrophils, eosinophils or mast cells, at a preselected physiological site. The method comprises administering a dosage form comprising an effective amount of a chemokine peptide 3, a chemokine peptide 2, a variant thereof, a derivative thereof, a compound of formula (IV), a compound of formula (V), a compound of formula (VI), a compound of formula (VII), a compound of formula (VIII), a compound of formula (IX), a compound of formula (X), a compound of formula (XI), a compound of formula (XII), or a combination thereof, wherein the dosage form is linked, either covalently or noncovalently, to a targeting moiety. The targeting moiety binds to a cellular component at the preselected physiological site.

Moreover, it is also envisioned that an agent of the invention may be a targeting moiety, as some of the agents are selective chemokine inhibitors, rather than pan-chemokine inhibitors. For example, an agent of the invention, e.g., peptide 2, may be useful in the targeted delivery of an isotope or other cytotoxic molecule to red cells for the treatment of disorders such as erythroid leukemia, erythroid myelosis, polycythemia vera or other erythroid dysplasias. Similarly, an agent of the invention that specifically targets a particular cell type may be useful in diagnostics. Thus, these agents can be radiolabeled (Chianelli et al., Nucl. Med. Comm., 18, 437 (1997)), or labeled with any other detectable signal, such as those useful in diagnostic imaging (e.g., MRI and CAT) to image sites of inflammation in disorders like rheumatoid arthritis and diabetes mellitus (type I).

Also provided is a therapeutic method to augment an immune response. The method comprises administering to a mammal an immunogenic moiety and an amount of a chemokine peptide 2, a variant thereof, a derivative thereof, a compound of formula (IV), a compound of formula (V), a compound of formula (VI), a compound of formula (VII), a compound of formula (VIII), a compound of formula (IX), a compound of formula (X), a compound of formula (XI), a compound of formula (XII), or a combination thereof, wherein the amount is effective to augment the immune response of the mammal to the immunogenic moiety. Thus, the invention also provides a vaccine comprising an immunogenic moiety and an amount of a chemokine peptide 2, a variant thereof or a derivative thereof, a compound of formula (IV), a compound of formula (V), a compound of formula (VI), a compound of formula (VII), a compound of formula (VIII), a compound of formula (IX), a compound of formula (X), a compound of formula (XI), a compound of formula (XII), or a combination thereof. As used herein, an "immunogenic moiety" means an isolated or purified composition or compound (e.g., a purified virus preparation or a native or recombinant viral or bacterial antigen) that, when introduced into an animal, preferably a mammal, results in a humoral and/or cellular immune response by the animal to the composition or compound. Also provided are modified vaccines, wherein the immunogenic moiety is coupled to peptide 2, a variant or derivative thereof. Peptide 2 increases the binding of the modified vaccine to the red blood cell pool and blocks Duffy binding of chemokines and so prolongs the residency time of the vaccine in the circulation and decreases chemokine-induced activity, either of which result in an augmented antibody response. It is envisaged that the modified vaccine is delivered by the same routes as those used for unmodified immunogen (e.g., intravenous, intramuscular or orally).

The invention also provides a therapeutic method to prevent or inhibit asthma. The method comprises administering to a mammal in need of such therapy an effective amount of a chemokine peptide 3, a chemokine peptide 2, a variant

thereof, a derivative thereof, a compound of formula (IV), a compound of formula (V), a compound of formula (VI), a compound of formula (VII), a compound of formula (VIII), a compound of formula (IX), a compound of formula (X), a compound of formula (XI), a compound of formula (XII), or a combination thereof. As described hereinbelow in Example 12, a peptide of the invention inhibited cellular inflammation and IgE responses in the lung of mice exposed to ovalbumin. Preferably, in this embodiment of the invention, a therapeutic agent is administered to the upper and/or lower respiratory tract. Preferred peptides useful in this embodiment of the invention are chemokine peptides of RANTES, MCP-1 and MIP1α.

Further provided is a therapeutic method to prevent or inhibit viral, e.g., poxvirus, herpesvirus (e.g., *Herpesvirus samiri*), cytomegalovirus (CMV) or lentivirus, infection or replication. The method comprises administering to a mammal in need of such therapy an effective amount of a chemokine peptide 3, a chemokine peptide 2, a variant thereof, a derivative thereof, a compound of formula (IV), or a compound of formula (V), a compound of formula (VI), a compound of formula (VII), a compound of formula (VIII), a compound of formula (IX), a compound of formula (X), a compound of formula (XI), a compound of formula (XII), or a combination thereof. Preferably, the therapeutic agents are employed to prevent or treat HIV. More preferably, the agent is administered before, during or after the administration of an anti-viral agent, e.g., for HIV AZT, a non-nucleoside reverse transcriptase inhibitor, a protease inhibitor or a combination thereof. It is also envisioned that a combination of a chemokine peptide 3, a chemokine peptide 2, a variant thereof, a derivative thereof, a compound of formula (IV), a compound of formula (V), a compound of formula (VI), a compound of formula (VII), a compound of formula (VIII), a compound of formula (IX), a compound of formula (X), a compound of formula (XI), or a compound of formula (XII) may be useful in the anti-viral methods and compositions of the invention. Preferred chemokine

peptides useful to prevent or inhibit viral infection are those from IP10, MIP1α, MIP1β, SDF-1, IL-8, GROα, RANTES and MCP-1.

A therapeutic method to prevent or treat low bone mineral density is also provided. The method comprises administering to a mammal in need of such therapy an effective amount of a chemokine peptide 3, a chemokine peptide 2, a variant thereof, a derivative thereof, a compound of formula (IV), a compound of formula (V), a compound of formula (VI), a compound of formula (VII), a compound of formula (VIII), a compound of formula (IX), a compound of formula (X), a compound of formula (XI), a compound of formula (XII), or a combination thereof. A preferred derivative of a variant of peptide 3 to prevent or treat low mineral bone density is CRD-$Cys_{13}Leu_4Ile_{11}$peptide 3(3-12)[MCP-1]. A preferred fragment of SEQ ID NO:1 useful in preventing or treating low mineral bone density is KQK.

Also provided is a method of suppressing tumor growth in a vertebrate animal comprising administering to said vertebrate a therapeutically effective amount of a chemokine peptide 3, a chemokine peptide 2, a variant thereof, a derivative thereof, a compound of formula (IV), a compound of formula (V), a compound of formula (VI), a compound of formula (VII), a compound of formula (VIII), a compound of formula (IX), a compound of formula (X), a compound of formula (XI), a compound of formula (XII), or a combination thereof. Preferably, the method increases or enhances macrophage, B cell-, T cell- or other immune cell-associated activity at a tumor site. A preferred peptide for use in this embodiment of the invention is a MCP-1 peptide.

Further provided is a method for preventing or treating rheumatoid arthritis in a mammal, comprising: administering to the mammal an effective amount of a chemokine peptide 3, a chemokine peptide 2, a variant thereof, a derivative thereof, a compound of formula (IV), a compound of formula (V), a compound of formula (VI), a compound of formula (VII), a compound of formula (VIII), a compound of formula (IX), a compound of formula (X), a compound of formula (XI), a compound

of formula (XII), or a combination thereof. For this embodiment of the invention, a preferred peptide is a MCP-1, MIP1α, MIP1β, GROα, and ENA78 peptide.

Also provided is a method to prevent or treat organ transplant rejection. The method comprises administering an effective amount of a chemokine peptide 3, a chemokine peptide 2, a variant thereof, a derivative thereof, a compound of formula (IV), a compound of formula (V), a compound of formula (VI), a compound of formula (VII), a compound of formula (VIII), a compound of formula (IX), a compound of formula (X), a compound of formula (XI), a compound of formula (XII), or a combination thereof.

Further provided is a method for preventing or treating psoriasis in a mammal, comprising: administering to the mammal an effective amount of a chemokine peptide 3, a chemokine peptide 2, a variant thereof, a derivative thereof, a compound of formula (IV), a compound of formula (V), a compound of formula (VI), a compound of formula (VII), a compound of formula (VIII), a compound of formula (IX), a compound of formula (X), a compound of formula (XI), a compound of formula (XII), or a combination thereof. Preferred peptides to prevent or treat psoriasis are peptides of MCP-1, RANTES, MIP1α, MIG, IP10, GROβ, GROα or MCP-3. A preferred derivative to prevent or treat psoriasis is a CRD-derivative of peptide 3.

Also provided is a method to enhance wound healing. The method comprises administering an effective amount of a chemokine peptide 3, a chemokine peptide 2, a variant thereof, a derivative thereof, a compound of formula (IV), a compound of formula (V), a compound of formula (VI), a compound of formula (VII), a compound of formula (VIII), a compound of formula (IX), a compound of formula (X), a compound of formula (XI), a compound of formula (XII), or a combination thereof.

Further provided is a method for preventing or treating an allergy in a mammal, comprising: administering to the mammal an effective amount of a chemokine peptide 3, a chemokine peptide 2, a variant thereof, a derivative thereof,

a compound of formula (IV), a compound of formula (V), a compound of formula (VI), a compound of formula (VII), a compound of formula (VIII), a compound of formula (IX), a compound of formula (X), a compound of formula (XI), a compound of formula (XII), or a combination thereof. Preferred peptides to prevent or treat allergies include peptides of RANTES, MIP1α, MCP-1, MCP-2, MCP-3, MCP-4, eotaxin or MIP1β.

Yet another embodiment of the invention is a method to prevent or inhibit an indication associated with elevated TNF-α. The method comprises administering an effective amount of a chemokine peptide 3, a chemokine peptide 2, a variant thereof, a derivative thereof, a compound of formula (IV), a compound of formula (V), a compound of formula (VI), a compound of formula (VII), a compound of formula (VIII), a compound of formula (IX), a compound of formula (X), a compound of formula (XI), a compound of formula (XII), or a combination thereof.

The invention also provides methods in which the nucleic acid molecules of the invention are administered to a mammal afflicted with, or at risk of, an indication associated with a chemokine-induced activity.

The invention also provides methods whereby the pharmacokinetics of desirable pharmaceutical agents may be modulated. In particular, agents which are normally rapidly cleared from the circulation may be retained longer by the addition of a peptide of the invention that has affinity for the Duffy antigen on red blood cells. This methodology may be particularly suited to modifying the pharmacokinetics of other biologically active, pharmaceutically useful peptides, as well as larger polypeptide or proteins. For example, a Duffy binding peptide (such as peptide 2[MCP-1] may be coupled or linked, either covalently or non-covalently, to a molecule such as recombinant human growth hormone (HGH) or insulin, and administered via a depo injection. By partitioning the modified HGH to the red blood cells, HGH may have much more suitable pharmacokinetics, with longer half times and less rapid changes in plasma concentrations. In another example, insulin coupled to a peptide of the invention may be particularly useful as a treatment for

the highly insulin-resistant type II diabetic whose disease has progressed significantly. It is also envisioned that other small molecules may be coupled to Duffy binding molecules in a manner which preserves the intended function of the active molecule and of the Duffy binding molecule. For coupling to recombinant proteins and peptides, Duffy binding peptides are preferred. For coupling to small molecule drugs, analogs (e.g., isosteres) of Duffy binding peptides are preferred.

Brief Description of the Figures

Figure 1 is a schematic of the trans-well migration assay. In most experiments, the peptide (wavy line) is added to the upper well with about 50,000 cells (O). The upper and lower wells are separated by a 5 μm or 8 μm pore size PVP-free membrane (- - - -). Chemokine (●) is added to the lower well. After 4 hours, the number of cells that have migrated through the membrane are measured (O in lower well).

Figure 2 shows a dose-response curve for the peptide 3 (SEQ ID NO:1) inhibition of MCP-1-induced THP-1 cell migration.

Figure 3 shows the reverse transcriptase activity present in the culture medium at day 21 after infection of Jurkat cells with a T-tropic HIV. Peptides were added on day 0, one hour prior to infection of the cells with HIV isolate. The full length chemokine SDF-1α was used as a positive control.

Figure 4 shows the structure of CRD-$Cys_{13}leu_4ile_{11}$peptide 3[MCP-1](3-12)[MCP-1], which is cyclized via disulphide bonds. The main chain α carbons are indicated by C_D which indicates that the D form of the amino acid is present.

Figure 5 depicts a schematic of inhibition of cell migration via binding of a therapeutic agent of the invention to a chemokine receptor. C^RC = a therapeutic agent of the invention. Chemokine receptors are shown as blackened rectangles.

Figure 6 depicts a schematic of the inhibition of HIV entry by an agent of the invention.

Figure 7 shows the dose-dependent inhibition of inflammation (A) and endotoxemia (B) in animal models by peptide 3 (CRD-$Cys_{13}Leu_4Ile_{11}$ peptide 3(3-12) [MCP-1] = NR58-3.14.3).

Figure 8 shows a preferred analog of peptide WVQ.

Figure 9 shows a graph of the number of macrophage at the site of LPS administration in a rat in the presence or absence of a peptide of the invention.

Figure 10 shows a graph of the number of B cells at the site of LPS administration in a rat in the presence or absence of a peptide of the invention.

Figure 11 shows a graph of the fraction of HIV infected THP-1 cells in the presence of peptide 2 or peptide 3 using a quantitative immunofluorescent (QIF) assay.

Figure 12 depicts codons for various amino acids.

Figure 13 depicts exemplary amino acid substitutions.

Figure 14 shows exemplary therapeutic agents of the invention.

Figure 15 shows the Duffy binding affinity and inhibition of THP-1 migration for selected peptide 2 compounds. LFL = linear forward L isomer; LRD = linear reverse D isomer; CRD = cyclic reverse D isomer; CFL = cyclic forward L isomer.

Figure 16 summarizes binding and ED_{50} data for selected peptides of the invention.

Figure 17 shows an exemplary protocol to test agents in a rat dermal inflammation model (CRD-$Cys_{13}Leu_4Ile_{11}$ peptide 3(3-12)[MCP-1] = NR58-3.14.3).

Detailed Description of the Invention

Definitions

"Chemokines" refers to a family of proinflammatory signaling molecules which act on macrophage, B cells, T cells, neutrophils, eosinophils, basophils, mast cells, smooth muscle cells, e.g., vascular smooth muscle cells, and the like (e.g., by affecting their migration, proliferation, or degranulation, or the immunomodulation

WHAT IS CLAIMED IS:

1. Chemokine peptide 3, a variant, or a derivative thereof.

2. Chemokine peptide 2, a variant, or a derivative thereof.

3. The peptide of claim 1 wherein the chemokine is not IL8 or NAP-2.

4. The peptide of claim 1 which is a variant of peptide 3[MCP-1].

5. The peptide of claim 4 which is Leu_4Ile_{11}peptide 3(3-12)[MCP-1].

6. The peptide of claim 1 or 2 which is a CC chemokine.

7. The peptide of claim 6 wherein the CC chemokine is MCP-1, RANTES, MCP-2, MCP-3, MCP-4, eotaxin, MIP1α, MIP1β, LARC, I309, HCC-1, TARC or Ckβ8.

8. The peptide of claim 1 or 2 which is a CXC chemokine.

9. The peptide of claim 8 wherein the CXC chemokine is IP-10, PF-4, SDF-1, NAP-2, GROα, GROβ, GROγ or ENA78.

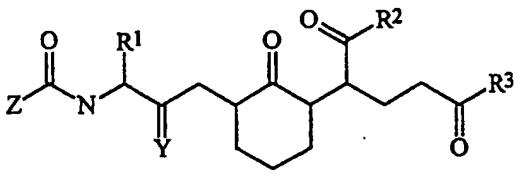
10. The peptide of claim 8 wherein the CXC chemokine is IL-8, IP-10, SDF-1, PF-4, NAP-2, GROα, GROβ, GROγ, NAP-2 or ENA78.

11. A CRD derivative of chemokine peptide 3 or a variant thereof.

12. The derivative of claim 11 which is CRD-$Cys_{13}Leu_4Ile_{11}$peptide 3(3-12)[MCP-1].

13. A CRD derivative of chemokine peptide 2 or a variant thereof.

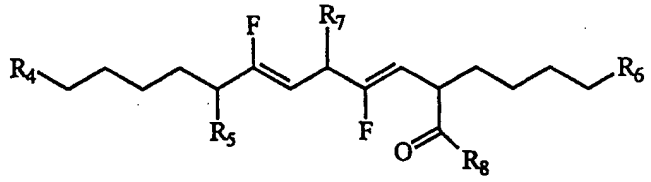
14. A compound of formula (IV):



(IV)

wherein R^1 is aryl, heteroaryl, coumaryl or chromanyl; wherein R^2 is $N(R^a)(R^b)$; wherein R^3 is $N(R^c)(R^d)$; wherein Y is oxo or thioxo; wherein Z is $(C_1\text{-}C_{10})$alkyl; wherein $R^a\text{-}R^d$ are each independently hydrogen, $(C_1\text{-}C_{10})$alkyl, $(C_1\text{-}C_{10})$alkanoyl, phenyl, benzyl or phenethyl; or wherein R^a and R^b, or R^c and R^d, together with the nitrogen to which they are attached form a pyrrolidino, piperidino or morpholino ring; or a pharmaceutically acceptable salt thereof.

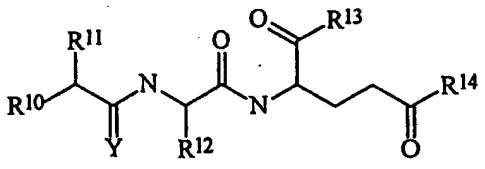
15. A compound of formula (V):



wherein R^4 is NR_kR_l; wherein R^5 is NR_mR_n; wherein R^6 is NR_oR_p; wherein R^7 is NR_qR_r; wherein R^8 is hydrogen, hydroxy, $(C_1\text{-}C_{10})$alkyl, $(C_3\text{-}C_6)$cycloalkyl,

$(C_3$-$C_6)$cycloalkyl$(C_1$-$C_6)$alkyl, $(C_1$-$C_{10})$alkoxy, $(C_3$-$C_6)$cycloalkyl$(C_1$-$C_6)$alkoxy, NR_sR_t, the N-terminal residue of an amino acid or a peptide of 2 to about 25 amino acid residues; wherein R_k, R_l, R_o, and R_p are each hydrogen; wherein R_m are R_n are each independently hydrogen, acetyl, $(C_1$-$C_{10})$alkyl, $(C_3$-$C_6)$cycloalkyl, propoxy, butoxy, *tert*-butoxycarbonyl, 9-fluorenylmethoxycarbonyl, the C-terminal residue of an amino acid or a peptide of 2 to about 25 amino acid residues; wherein R_q and R_r are each independently hydrogen, $(C_1$-$C_{10})$alkyl, or $(C_3$-$C_6)$cycloalkyl; and wherein R_s are R_t are each independently hydrogen, $(C_1$-$C_{10})$alkyl, $(C_3$-$C_6)$cycloalkyl, $(C_3$-$C_6)$cycloalkyl$(C_1$-$C_6)$alkyl, phenyl, benzyl, or phenethyl; or a pharmaceutically acceptable salt thereof.

16. A compound of formula (VI):



(VI)

wherein R^{10} is NR^iR^j; R^{11} is aryl, heteroaryl, aryl$(C_1$-$C_3)$alkyl, heteroaryl$(C_1$-$C_3)$alkyl, coumaryl, coumaryl$(C_1$-$C_3)$alkyl, chromanyl or chromanyl$(C_1$-$C_3)$alkyl; wherein any aryl or heteroaryl group, or the benz-ring of any coumaryl or chromanyl group may optionally be substituted with one, two or three substituents selected from the group consisting of halo, nitro, cyano, hydroxy, $(C_1$-$C_6)$alkyl, $(C_1$-$C_6)$alkoxy, $(C_1$-$C_6)$alkanoyl, $(C_2$-$C_6)$alkanoyloxy, -C(=O)$(C_1$-$C_6)$alkoxy, C(=O)NR^gR^h, NR^eR^f; R^{12} is $(C_1$-$C_6)$alkyl; R^{13} is $(C_1$-$C_{10})$alkyl, $(C_3$-$C_6)$cycloalkyl, $(C_3$-$C_6)$cycloalkyl$(C_1$-$C_6)$alkyl, $(C_1$-$C_{10})$alkoxy, $(C_3$-$C_6)$cycloalkyl$(C_1$-$C_6)$alkoxy, hydroxy, or $N(R^a)(R^b)$; R^{14} is $(C_1$-$C_{10})$alkyl, $(C_3$-$C_6)$cycloalkyl, $(C_3$-$C_6)$cycloalkyl$(C_1$-$C_6)$alkyl, $(C_1$-$C_{10})$alkoxy, $(C_3$-$C_6)$cycloalkyl$(C_1$-$C_6)$alkoxy or $N(R^c)(R^d)$; Y is oxo or thioxo; and wherein R^a-R^j are each independently hydrogen, $(C_1$-$C_{10})$alkyl, $(C_1$-$C_{10})$alkanoyl,

phenyl, benzyl, or phenethyl; or R^a and R^b, R^c and R^d, R^e and R^f, R^g and R^h, or R^i and R^j together with the nitrogen to which they are attached form a ring selected from pyrrolidino, piperidino, or morpholino; or a pharmaceutically acceptable salt thereof.

17. A method of preventing or inhibiting an indication associated with a chemokine-induced activity, comprising: administering to a mammal afflicted with, or at risk of, the indication an amount of a chemokine peptide 3, a chemokine peptide 2, a variant thereof, a derivative thereof, or a combination thereof, effective to prevent or inhibit said activity, wherein the chemokine is not IL8 or NAP-2.

18. A method to inhibit the activity of more than one chemokine, comprising: administering to a mammal in need thereof an effective amount of a chemokine peptide 3, a chemokine peptide 2, a variant thereof, a derivative thereof, a compound of formula (IV), a compound of formula (V), a compound of formula (VI), or a combination thereof.

19. A method to increase or enhance a chemokine-associated inflammatory response in a mammal, comprising: administering to the mammal an amount of a chemokine peptide 2, a variant thereof, a derivative thereof, or a combination thereof effective to increase or enhance said response.

20. A method of preventing or inhibiting an indication associated with hematopoietic cell recruitment, comprising: administering to a mammal at risk of, or afflicted with, the indication an effective amount of a chemokine peptide 3, a chemokine peptide 2, a variant thereof, a derivative thereof, a compound of formula (IV), a compound of formula (V), a compound of formula (VI), or a combination thereof.

21. A method of preventing or inhibiting an indication associated with histamine release from basophils or mast cells, comprising administering to a mammal at risk of, or afflicted with, the indication an effective amount of a chemokine peptide 3, a chemokine peptide 2, a variant thereof, a derivative thereof, a compound of formula (IV), a compound of formula (V), a compound of formula (VI), or a combination thereof.

22. A method to modulate the chemokine-induced activity of hematopoietic cells at a preselected physiological site, comprising: administering to a mammal a dosage form comprising an effective amount of a chemokine peptide 3, a chemokine peptide 2, a variant thereof, a derivative thereof, a compound of formula (IV), a compound of formula (V), a compound of formula (VI), or a combination thereof, wherein the dosage form is linked to a site targeting moiety.

23. A method to augment an immune response, comprising: administering to a mammal an immunogenic moiety and an amount of a chemokine peptide 2, a variant thereof, a derivative thereof, a compound of formula (IV), a compound of formula (V), a compound of formula (VI), or a combination thereof, wherein the amount is effective to augment the immune response of the mammal to the immunogenic moiety.

24. A therapeutic method to prevent or treat a vascular indication, comprising: administering to a mammal in need of such therapy an effective amount of a chemokine peptide 3, a chemokine peptide 2, a variant thereof, a derivative thereof, a compound of formula (IV), a compound of formula (V), a compound of formula (VI), or a combination thereof, wherein the indication is coronary artery disease, myocardial infarction, unstable angina pectoris, atherosclerosis or vasculitis.

25. A therapeutic method to prevent or inhibit lentiviral infection or replication, comprising: administering to a mammal in need of such therapy an effective amount of a chemokine peptide 3, a chemokine peptide 2, a variant thereof, a derivative thereof, a compound of formula (IV), a compound of formula (V), a compound of formula (VI), or a combination thereof.

26. The method of claim 25 wherein the lentivirus is HIV.

27. The method of claim 26 further comprising administering an antiviral agent before, during and/or after the administration of the peptide, a variant thereof, derivative thereof, the compound of formula (IV), the compound of formula (V), the compound of formula (VI), or a combination thereof.

28. A therapeutic method to prevent or treat low bone mineral density, comprising: administering to a mammal in need of such therapy an effective amount of a chemokine peptide 3, a chemokine peptide 2, a variant thereof, a derivative thereof, a compound of formula (IV), a compound of formula (V), a compound of formula (VI), or a combination thereof.

29. A method of inhibiting a parasitic infection in a vertebrate animal, comprising: administering to the animal an effective amount of a chemokine peptide 2, a variant thereof, a derivative thereof, or a combination thereof.

30. The method of claim 29 wherein the animal is a human with malaria.

31. A therapeutic method to prevent or treat an autoimmune disease, comprising: administering to a mammal in need of such therapy an effective amount of a chemokine peptide 3, a chemokine peptide 2, a variant thereof, a derivative

thereof, a compound of formula (IV), a compound of formula (V), a compound of formula (VI), or a combination thereof.

32. A method of suppressing tumor growth in a vertebrate animal, comprising: administering to said vertebrate an effective amount of a chemokine peptide 3, a chemokine peptide 2, a variant thereof, a derivative thereof, a compound of formula (IV), a compound of formula (V), a compound of formula (VI), or a combination thereof.

33. A method for preventing or treating psoriasis in a mammal, comprising: administering to the mammal an effective amount of a chemokine peptide 3, a chemokine peptide 2, a variant thereof, a derivative thereof, a compound of formula (IV), a compound of formula (V), a compound of formula (VI), or a combination thereof.

34. A method to increase or enhance hematopoietic cell-associated activity at a tumor site, comprising: administering an effective amount of a chemokine peptide 3, a chemokine peptide 2, a variant thereof, a derivative thereof, a compound of formula (IV), a compound of formula (V), a compound of formula (VI), or a combination thereof.

35. A method to enhance wound healing, comprising: administering an effective amount of a chemokine peptide 3, a chemokine peptide 2, a variant thereof, a derivative thereof, a compound of formula (IV), a compound of formula (V), a compound of formula (VI), or a combination thereof.

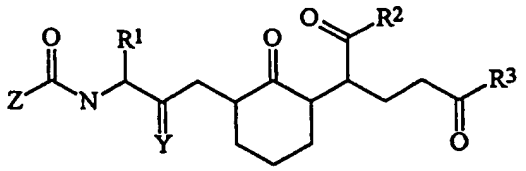
36. A method of treating a mammal afflicted with, or at risk of, an indication associated with chemokine-induced activity, comprising: administering to the mammal an effective amount of a compound of formula (IV):

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Further defect(s) under Article 17(2)(a):

Claims Nos.: 1,3,4,6-11,17,18,20-22,24-28,31-35,40-46,48-51,55(all partially)

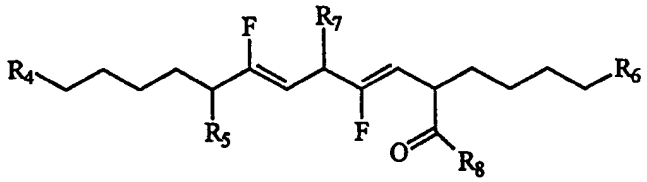
The definition of "chemokine peptide 3" is vague and ambiguous. Several different definitions have been used to define this expression: e.g., see Table 1 on page 129, showing a peptide of 12 amino acids; page 31, line 25 to page 32, describing this expression as a peptide of 3-30 amino acids originating from the C-terminal part of any chemokine and also the definitions on the pages 3-5 relating to facultatively repeating sequences of amino acids of a general character lacking any significant structural constant entity. Therefore said expression "chemokine peptide 3" cannot be considered to be a clear and concise definition of patentable subject-matter (Art.6 PCT).
Furthermore the available experimental data actually only comprise a very small part of the compounds claimed, which part is moreover not evenly distributed over the whole claimed area. Therefore the claims can also not be considered to represent a permissible generalisation which is fairly based on experimental evidence, that is, they are also not adequately supported by the description (Art.6 PCT).
Therefore a meaningful and economically feasible search could not encompass the complete subject-matter of subject 1. Consequently the search has been limited to the actually synthesised compounds of the examples 1 and 2 and (partially) Figure 14 and the relevant SEQ ID's encompassed by subject 1 and their use and has been extended to compounds having the same activity (Art.17(2)(a)(ii) and (b) PCT, PCT Guidelines CIII,2.1 and CIII,3,7).



(IV)

wherein R^1 is aryl, heteroaryl, coumaryl or chromanyl; wherein R^2 is $N(R^a)(R^b)$; wherein R^3 is $N(R^c)(R^d)$; wherein Y is oxo or thioxo; wherein Z is $(C_1\text{-}C_{10})$alkyl; wherein $R^a\text{-}R^d$ are each independently hydrogen, $(C_1\text{-}C_{10})$alkyl, $(C_1\text{-}C_{10})$alkanoyl, phenyl, benzyl or phenethyl; or wherein R^a and R^b, or R^c and R^d, together with the nitrogen to which they are attached form a pyrrolidino, piperidino or morpholino ring; or a pharmaceutically acceptable salt thereof.

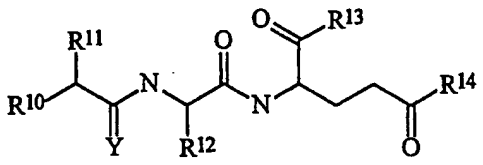
37. A method of treating a mammal afflicted with, or at risk of, an indication associated with chemokine-induced activity, comprising: administering to the mammal an effective amount of a compound of formula (V):



wherein R^4 is NR_kR_l; wherein R^5 is NR_mR_n; wherein R^6 is NR_oR_p; wherein R^7 is NR_qR_r; wherein R^8 is hydrogen, hydroxy, $(C_1\text{-}C_{10})$alkyl, $(C_3\text{-}C_6)$cycloalkyl, $(C_3\text{-}C_6)$cycloalkyl$(C_1\text{-}C_6)$alkyl, $(C_1\text{-}C_{10})$alkoxy, $(C_3\text{-}C_6)$cycloalkyl$(C_1\text{-}C_6)$alkoxy, NR_sR_t, the N-terminal residue of an amino acid or a peptide of 2 to about 25 amino acid residues; wherein R_k, R_l, R_o, and R_p are each hydrogen; wherein R_m are R_n are each independently hydrogen, acetyl, $(C_1\text{-}C_{10})$alkyl, $(C_3\text{-}C_6)$cycloalkyl, propoxy, butoxy,

tert-butoxycarbonyl, 9-fluorenylmethoxycarbonyl or the C-terminal residue of an amino acid or a peptide of 2 to about 25 amino acid residues; wherein R_q and R_r are each independently hydrogen, $(C_1\text{-}C_{10})$alkyl, or $(C_3\text{-}C_6)$cycloalkyl; and wherein R_s are R_t are each independently hydrogen, $(C_1\text{-}C_{10})$alkyl, $(C_3\text{-}C_6)$cycloalkyl, $(C_3\text{-}C_6)$cycloalkyl$(C_1\text{-}C_6)$alkyl, phenyl, benzyl, or phenethyl; or a pharmaceutically acceptable salt thereof.

38. A method of treating a mammal afflicted with, or at risk of, an indication associated with chemokine-induced activity, comprising: administering to the mammal an effective amount of a compound of formula (VI):



(VI)

wherein R^{10} is NR^iR^j; R^{11} is aryl, heteroaryl, aryl$(C_1\text{-}C_3)$alkyl, heteroaryl$(C_1\text{-}C_3)$alkyl, coumaryl, coumaryl$(C_1\text{-}C_3)$alkyl, chromanyl or chromanyl$(C_1\text{-}C_3)$alkyl; wherein any aryl or heteroaryl group, or the benz-ring of any coumaryl or chromanyl group may optionally be substituted with one, two or three substituents selected from the group consisting of halo, nitro, cyano, hydroxy, $(C_1\text{-}C_6)$alkyl, $(C_1\text{-}C_6)$alkoxy, $(C_1\text{-}C_6)$alkanoyl, $(C_2\text{-}C_6)$alkanoyloxy, $-C(=O)(C_1\text{-}C_6)$alkoxy, $C(=O)NR^gR^h$, NR^eR^f; R^{12} is $(C_1\text{-}C_6)$alkyl; R^{13} is $(C_1\text{-}C_{10})$alkyl, $(C_3\text{-}C_6)$cycloalkyl, $(C_3\text{-}C_6)$cycloalkyl$(C_1\text{-}C_6)$alkyl, $(C_1\text{-}C_{10})$alkoxy, $(C_3\text{-}C_6)$cycloalkyl$(C_1\text{-}C_6)$alkoxy, hydroxy, or $N(R^a)(R^b)$; R^{14} is $(C_1\text{-}C_{10})$alkyl, $(C_3\text{-}C_6)$cycloalkyl, $(C_3\text{-}C_6)$cycloalkyl$(C_1\text{-}C_6)$alkyl, $(C_1\text{-}C_{10})$alkoxy, $(C_3\text{-}C_6)$cycloalkyl$(C_1\text{-}C_6)$alkoxy or $N(R^c)(R^d)$; Y is oxo or thioxo; and wherein $R^a\text{-}R^j$ are each independently hydrogen, $(C_1\text{-}C_{10})$alkyl, $(C_1\text{-}C_{10})$alkanoyl, phenyl, benzyl, or phenethyl; or R^a and R^b, R^c and R^d, R^e and R^f, R^g and R^h, or R^i and R^j together with the nitrogen to which they are attached form a ring

selected from pyrrolidino, piperidino, or morpholino; or a pharmaceutically acceptable salt thereof.

39. An immunogenic composition comprising an immunogenic moiety and an amount of a chemokine peptide 2, a variant thereof, a derivative thereof, a compound of formula (IV), a compound of formula (V), a compound of formula (VI), or a combination thereof.

40. A therapeutic method to prevent or treat asthma, comprising: administering to a mammal in need of such therapy an effective amount of a chemokine peptide 3, a chemokine peptide 2, a variant thereof, a derivative thereof, a compound of formula (IV), a compound of formula (V), a compound of formula (VI), or a combination thereof.

41. The method of claim 17 wherein the amount inhibits a product or intermediate in the arachidonic acid pathway.

42. The method of claim 41 wherein leukotriene is inhibited.

43. The method of claim 41 wherein thromboxane is inhibited.

44. The method of claim 41 wherein prostaglandin is inhibited.

45. A method of preventing or inhibiting an indication associated with elevated TNF-α, comprising: administering to a mammal afflicted with, or at risk of, the indication an effective amount of a chemokine peptide 3, ā chemokine peptide 2, a variant thereof, a derivative thereof, a compound of formula (IV), a compound of formula (V), a compound of formula (VI) or a combination thereof.

46. A peptide that includes the amino acid sequence KXK, which is a chemokine antagonist, activates TGF-beta, or a combination thereof.

47. CRD-$Cys_{13}Leu_4Ile_{11}$peptide 3(3-12)[MCP-1].

48. A therapeutic method to prevent or treat organ transplant rejection, comprising: administering to a mammal in need of such therapy an effective amount of a chemokine peptide 3, a chemokine peptide 2, a variant thereof, a derivative thereof, a compound of formula (IV), a compound of formula (V), a compound of formula (VI), or a combination thereof.

49. A therapeutic method to prevent or treat rheumatoid arthritis, comprising: administering to a mammal in need of such therapy an effective amount of a chemokine peptide 3, a chemokine peptide 2, a variant thereof, a derivative thereof, a compound of formula (IV), a compound of formula (V), a compound of formula (VI), or a combination thereof.

50. A therapeutic method to prevent or treat allergy, comprising: administering to a mammal in need of such therapy an effective amount of a chemokine peptide 3, a chemokine peptide 2, a variant thereof, a derivative thereof, a compound of formula (IV), a compound of formula (V), or a compound of formula (VI), or a combination thereof.

51. Use of a chemokine peptide 3, a chemokine peptide 2, a variant thereof, a derivative thereof, a compound of formula (IV), a compound of formula (V), or a compound of formula (VI), or a combination thereof for the manufacture of a medicament for the treatment of a pathological condition or symptom in a mammal which is associated with a chemokine-induced activity.

52. A compound of formula (IV) for use in medical therapy.

53. A compound of formula (V) for use in medical therapy.

54. A compound of formula (VI) for use in medical therapy.

55. A chemokine peptide 3, a variant or derivative thereof for use in medical therapy.

56. A chemokine peptide 2, a variant or derivative thereof for use in medical therapy.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 98/19052

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C07K14/52 A61K38/19 C07K5/08 C07C237/22 C07C229/30
C07K7/64 A61K45/06

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07K C07C A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5 079 228 A (COHEN ALLEN B ET AL) 7 January 1992 see the whole document ---	1,3-10, 17,18, 20-22, 24-28, 31-35, 40-46, 48-51,55
X	WO 94 20512 A (GLYCAN PHARM INC) 15 September 1994 See especially example 9, figure 4 and claims --- -/--	1,3-10, 17,18, 20-22, 24-28, 31-35, 40-46, 48-51,55

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
3 March 1999	11 06 1999

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	GROENENDIJK, M

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/US 98/19052

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 5079228 A	07-01-1992	NONE	
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		AU 6353294 A	26-09-1994
		CA 2157388 A,C	15-09-1994
		EP 0688327 A	27-12-1995
		EP 0900804 A	10-03-1999
		US 5795860 A	18-08-1998
WO 8604334 A	31-07-1986	AU 602483 B	18-10-1990
		AU 5319886 A	13-08-1986
		EP 0215805 A	01-04-1987
		JP 62501502 T	18-06-1987
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		CA 2091558 A	13-03-1992
		EP 0550528 A	14-07-1993
		PT 98935 A	31-07-1992
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WO 9724325 A	10-07-1997	AU 1208397 A	28-07-1997
		JP 10081665 A	31-03-1998

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 86 04334 A (MERCK PATENT GMBH) 31 July 1986 see claims 1-16; tables 1,4,7	1,3,4,6, 7,39,55
X	WO 92 04372 A (SCRIPPS RESEARCH INST) 19 March 1992 see claim 1	1,8,10
X	THOMPSON E.A.: "Design and evaluation of small peptides mapping the exposed surface of IL-8" INTERNATIONAL JOURNAL OF PEPTIDE AND PROTEIN RESEARCH, vol. 47, 1996, COPENHAGEN DK, pages 214-218, XP002095213 see the whole document	1,8,10
X	CHEMICAL ABSTRACTS, vol. 82, no. 11, 17 March 1975 Columbus, Ohio, US; abstract no. 73463, NAGAMATSU E.A.: "Hydrolysis of lysine peptides by plasmin" XP002095217 see abstract & CHEM.PHARM.BULL., vol. 22, no. 11, 1974, pages 2680-2684,	1,3,6,7
X	CHEMICAL ABSTRACTS, vol. 82, no. 13, 31 March 1975 Columbus, Ohio, US; abstract no. 84651, NOGUCHI: "Isolation and identification of acidic oligopeptides..." XP002095218 see abstract & J.ARIC.FOOD CHEM., vol. 23, no. 1, - 1975 pages 49-53,	1,3,6,7
X	DATABASE WPI Section Ch, Week 9519 Derwent Publications Ltd., London, GB; Class B04, AN 95-143871 XP002095220 -& JP 07 067 689 A (ZH KAGAKU & KESSEI RYOHO KENKYUSHO) , 14 March 1995 see page 1424	1,3,6,7

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	CHEMICAL ABSTRACTS, vol. 103, no. 17, 28 October 1985 Columbus, Ohio, US; abstract no. 137328, PORSCHKE E.A: "The configuration of single stranded oligonucleotides..." XP002095219 see abstract & J.BIOL.STRUCT.DYN., vol. 2, no. 6, 1985, pages 1173-1184, ---	1,3,8,9
P,X	WO 98 12324 A (CENTER FOR BLOOD RESEARCH) 26 March 1998 The whole document; see especially SEQ ID NO. 10 ---	1,3-10, 17,18, 20-22, 24-28, 31-35, 40-46, 48-51,55
A	WELLS E.A.: "The molecular basis of the chemokine/chemokine receptor interaction-scope for design of chemokine antagonists." METHODS: A COMPANION TO METHODS OF ENZYMOLOGY, vol. 10, 1996, pages 126-134, XP002095214 see the whole document ---	1,3-12, 17,18, 20-22, 24-28, 31-35, 40-51,55
A	GONG J H ET AL: "Antagonists of monocyte chemoattractant protein 1 identified by modification of functionally critical NH2-terminal residues." JOURNAL OF EXPERIMENTAL MEDICINE, (1995 FEB 1) 181 (2) 631-40. JOURNAL CODE: I2V. ISSN: 0022-1007., UNITED STATES, XP000605169 see the whole document ---	
A	WO 97 24325 A (TAKEDA CHEMICAL INDUSTRIES LTD ;KATO KANEYOSHI (JP); YAMAMOTO MITS) 10 July 1997 see the whole document ---	
A	SIMMONS E.A.: "Potent inhibition of HIV-1 infectivity in macrophages and lymphocytes by a novel CCR5 antagonist" SCIENCE, vol. 276, 11 April 1997, pages 276-279, XP002095215 see the whole document ---	

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INTERNATIONAL SEARCH REPORT

Inten ...nal Application No
PCT/US 98/19052

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	GONG J H ET AL: "An antagonist of monocyte chemoattractant protein 1 (MCP -1) inhibits arthritis in the MRL-lpr mouse model." JOURNAL OF EXPERIMENTAL MEDICINE, (1997 JUL 7) 186 (1) 131-7. JOURNAL CODE: I2V. ISSN: 0022-1007., UNITED STATES, XP002095216 see the whole document -----	

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Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

I. national application No.
PCT/US 98/19052

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
Remark: Although claims 17-38, 40-45 and 48-50 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [X] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
see FURHTER INFORMATION sheet

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:
see FURTHER INFORMATION sheet

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [X] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
see FURTHER INFORMATION sheet, subject 1.

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 1,3-5,11,12,47,55(complete);6-10,17,18,20-22,24-28, 31-35,40-46,48-51(partially)

   Chemokine peptide 3, a variant or derivative thereof and their medical use.

2. Claims: 2,13,19,29,30,56(complete),6-10,17,18,20-28,31-35, 39-45,48-51(partially)

   Chemokine peptide 2, a variant or derivative thereof and their medical use.

3. Claims: 14,36,52(complete),18,20-28,31-35,39,40,45, 48-51(partially)

   A compound of formula IV and its medical use

4. Claims: 15,37,53(complete),18,20-28,31-35,39,40,45, 48-51(partially)

   A compound of formula V and its medical use

5. Claims: 16,38,54(complete),18,20-28,31-35,39,40,45, 48-51(partially)

   A compound of formula VI and its medical use

6. Claim : 46(partially)

   Peptides as defined in claim 46 as far as not encompassed by subject 1 and their medical use.